109. A transformed maize seed, the endosperm of which contains an elevated level of lysine or a sulfur-containing amino acid compared to a corresponding non-transformed maize seed.

110. A transformed maize seed which has been transformed with a plant derived polynucleotide to express a polypeptide in the endosperm of the transformed maize seed, wherein the transformed maize seed exhibits an elevated level of lysine or a sulfur-containing amino acid compared to a corresponding non-transformed maize seed.

111. A food or feed product produced from the transformed cereal plant seed of claim 109.--

REMARKS

Reconsideration of the present application is respectfully requested.

Claims 75-94 are in the application for consideration. Claims 75, 80-89, and 94 have been cancelled. Claims 95-111 have been added. The independent claims have been rewritten to require the polynucleotide is a "plant derived polynucleotide". Support for the term is found throughout the specification and in particular on page 15, line 4. The applicant submits that "seed storage protein" does not cover many of the proteins suitable for practicing the invention, such as chymotrypsin inhibitor and hordothionin protein.

Claim 94 is objected to because at line 3, "and" should be changed to --an--. The Examiner's observation of the typo is noted with appreciation. The claim has been rewritten as claim 106.

Claims 75-94 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are also rejected under 35 U.S.C. 112, first paragraph, because the specification is enabling only for claims limited to transformed cereal plant seed

having an elevated lysine, methionine, and cysteine content (about 10% to about 35% by weight compared to untransformed cereal plant seed) comprising the modified hordothionin gene of SEQ ID NO:2 (HT12)....

The Examiner indicates the rejections are repeated for the reasons of record set forth in the Official action mailed 5/18/99 as applied to Claims 1-21, the Official action mailed 11/22/99 as applied to Claims 6,7, 14-17, and 21-35, the Official action mailed 4/21/00 as applied to Claims 36-56, and the Official action mailed 8/9/00 as applied to Claims 57-74.

As claims 1-74 are no longer in the application for consideration, the Applicant will attempt to address the issues as they apply to the current claims. If there are issues that Applicant has not addressed, the Examiner is requested to clarify and point out these issues in a subsequent Office action.

In the Official action mailed 4/21/00 the Examiner referenced the Official action mailed 5/18/99 and 11/22/99 and made no further explanation of the rejection.

In the Official action mailed 11/22/99 the Examiner states that the Applicant's description of appropriate substitution of amino acids is limited to the hordothionin protein. The Examiner further states that the Applicant only generally describes amino acid substitution for other proteins, and detailed description with respect to ESA is not provided in the as-filed specification.

The rejection is respectfully traversed. Pages 12-13 of the present specification describe suitable polypeptides other than hordothionin and hordothionin derivatives. With regard to ESA, a pending application was incorporated by reference. The ESA application is now a patent and the present application has been amended to reflect that information.

It is noted that amino acid substitution is not required for all plant derived polypeptides. Many plant derived polypeptides are already rich in lysine or a sulfur-containing amino acid and are disclosed in the present application. The polypeptides from the application are listed below for the Examiner's convenience.

"Many other proteins are also appropriate, for example the protein encoded by the structural gene can be a lysine and/or sulfur rich seed protein, such as the soybean 2S albumin described in U.S. Ser. No. 08/618,911 filed March 20, 1996,

and the chymotrypsin inhibitor from barley, Williamson et al., Eur. J Biochem 165: 99-106 (1987), the disclosures of each are incorporated by reference.

Derivatives of these genes can be made by site directed mutagenesis to increase the level of preselected amino acids in the encoded polypeptide. For example the gene encoding for the barley high lysine polypeptide (BHL), is derived from barley chymotrypsin inhibitor, U.S. Ser. No. 08/740,682 filed November 1, 1996 and PCT/US97/20441 filed October 31, 1997, the disclosures of each are incorporated herein by reference. The gene encoding for the enhanced soybean albumin gene (ESA) , is derived from soybean 2S albumin described in U.S. Ser. No. 08/618,911, the disclosure of which is incorporated herein in its entirety by reference.

Other examples of sulfur-rich plant proteins within the scope of the invention include plant proteins enriched in cysteine but not methionine, such as the wheat endosperm purothionine (Mak and Jones; Can. J. Biochem.; Vol. 22; p. 83J; (1976); incorporated herein in its entirety by reference), the pea low molecular weight albumins (Higgins et al.; J. Biol. Chem.; Vol. 261; p. 11124; (1986); incorporated herein in its entirety by reference) as well as 2S albumin genes from other organisms. See, for example, Coulter et al.; J. Exp. Bot.; Vol. 41; p. 1541; (1990); incorporated herein in its entirety by reference.

Such proteins also include methionine-rich plant proteins such as from sunflower seed (Lilley et al.; In: Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs; Applewhite, H. (ed.); American Oil Chemists Soc.; Champaign, IL; pp. 497-502; (1989); incorporated herein in its entirety by reference), corn (Pedersen et al.; J. Biol. Chem. p. 261; p. 6279; (1986); Kirihara et al.; Gene, Vol. 71; p. 359; (1988); both incorporated herein in its entirety by reference), and rice (Musumura et al.; Plant Mol. Biol.; Vol. 12; p. 123; (1989); incorporated herein in its entirety by reference)."

In the 11/22/99 Office Action the Examiner further states that it is not the claimed subject matter that is relevant in the cited case law, but rather the concept that description of a single species does not adequately describe the genus when no predictions can be made for other members of the genus based on the described

species. The Examiner then states that it is maintained that Applicant has not provided appropriate written description of the genus, and it is not clear from the specification that Applicant was in possession of the invention as broadly claimed.

The statement is respectfully traversed. As noted above the Applicant has described and claimed numerous suitable plant proteins other than hordothionin. The Federal Register/Vol. 66, No. 4/Friday, January 5, 2001 provides "Guidelines for the Examination of Patent Applications Under the 35 U.S.C., 112 paragraph 1. The USPTO Guidelines state that to satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the invention had possession of the claimed invention. An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations.

It is respectfully submitted that Applicants have met this criteria. In addition to the many specific examples given in the present specification and recited above, suitable proteins are broadly described in the present specification. For example on page 6, line 21-page 7, line 4 describes high lysine proteins and high sulfur content proteins. One skilled in the art can readily determine other suitable plant proteins based on this written description.

In the Official action dated 5/18/99 the Examiner stated that the Applicant does not describe other transformed cereal plant seed modified in other amino acids transformed with other modified or unmodified genes, hence it is not clear from the instant specification that the Applicant was in possession of the invention as broadly claimed.

The Examiner indicates *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988) lists eight considerations for determining whether or not undue experimentation would be necessary to practice an invention. These factors are: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples of the invention, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims.

However, it is submitted that when determining the quantity of experimentation necessary, the focus is not on the amount of experimentation necessary to practice the entire genus, but the amount of experimentation required to practice any particular member. This concept is the central holding of *In re Wands* where the claims read on the use of any IgM antibody that possessed a particular binding affinity. This is similar to the present case where the claims read on increasing the level of lysine or sulfur-containing amino acid compared to a non-transformed plant which can be readily determined.

The *Wands* court recognized that it would require an infinite amount of experimentation to obtain every single possible IgM antibody that could be generated with the specified affinity. Accordingly, the court focused on the amount of experimentation necessary to practice any particular IgM antibody with the recited binding affinity and not the amount of experimentation required to practice the entire genus. This focus is further supported by the multitude of chemical patents that have issued with generic claims reading on tens to hundreds of thousands of individual members.

The question then becomes how much experimentation is required to create the claimed invention for increasing the level of lysine or sulfur-containing amino acid compared to a non-transformed plant. Applicants submit that no more than routine experimentation is required. This may be accomplished by the methods within the present application and within the technical, scientific, skill in the art.

Applicants assert the present invention is disclosed in a way that one skilled in the art will be able to practice it without an undue amount of experimentation. *In re Borkowski*, 422 F.2d 904, 908, 164 USPQ 642, 645 (CCPA 1970). Applicants submit that they have fully described the present invention as claimed by teaching both how to make and how to use the invention in a manner commensurate in scope with the claims.

The USPTO carries the initial burden to establish a reasonable basis for questioning the enablement provided for the claimed invention. As stated in *In re Wright*, 99, F.2d 1557, 27 USPQ2d 1510 (Fed. Cir. 1993); MPEP § 2164.04, the enablement requirement is satisfied if the specification describes any method for

making and using the claimed invention that bears a "reasonable correlation" to the entire scope of the claims. Applicants submit that this has been accomplished in the present application.

Further in the 5/18/99 Office action the Examiner indicates that the state of the art for amino acid substitution is highly unpredictable. In particular, the role of specific amino acids in protein function cannot be reliably predicted, and the effect of amino acid substitution on protein activity must be determined empirically. Furthermore, the state of the art for modification of gene expression or of phenotypic characteristics in plants by genetic transformation is highly unpredictable and hence significant guidance is required to practice the art without undue experimentation.

Amino acid substitution is only one method to obtain suitable polypeptides. As discussed above it is noted that native plant derived polypeptides can be selected that contain a high level of either high lysine or high level of sulfur-containing amino acids.

The Examiner also indicates that specific effects of given promoters, leaders, DNA sequences, and terminator sequences or gene expression in transformed plants can not be anticipated reliably and must be determined empirically.

It is submitted that the present specification has presented information that is pertinent in practicing the present claimed invention. In particular the claims call for endosperm-preferred promoters. The specification discloses such promoters. Suitable leaders, DNA sequences, and terminator sequences are known to those of skill in the art and are not critical to the present invention.

The Examiner further notes that in genetically modified plants, the introduced transgenes are sometimes not expressed, and they can also result in co-suppression effects. None of these effects are predictable, and the mechanism of gene silencing are still not fully understood. Moreover the phenotypic characteristics that will result from expression of a given DNA construct can not reliably be predicted. In fact, often the expected phenotypic result is not achieved. For example, antisense expression of polygalacturonase gene in transgenic tomato had no effect on fruit softening.

With regard to gene expression, transgenic events must always be screened for gene expression and the desired phenotype. This is an essential aspect of plant biotech research. The polygalacturonase example in no way diminishes the enablement of the present claims. The goal of the authors was antisense gene silencing of the polygalacturonase gene. The authors achieved their goal of silencing the gene. The authors state "However the physiological and biochemical changes that cause softening are complex and may not relate only to the pectin fraction". Therefore, other unidentified genes are likely involved in fruit softening.

In the present case the inventors have shown that expression in endosperm of polynucleotides and polypeptides derived from plants produces the desired benefit, i.e. increased amino acid content. Other genes are not required to produce the specific benefit.

Further in the 5/18/99 Office action the Examiner states the lack of guidance in the specification with regard to amino acid substitutions. Again it is emphasized that amino acid substitutions are not required. There are many suitable polypeptides that are listed above and in the application that do not require substitution. The Examiner also refers to the Wands factors which are discussed in detail above.

In the present Office action mailed 6/25/01 Claims 75-94 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner rejects Claim 75 stating that the phrase "nutritional value" is indefinite because it is not clear what is encompassed by the phrase. Claim 75 has been rewritten as claim 95 and is believed to overcome the objection.

The Examiner rejects Claim 75 with regard to the phrases "high lysine content" and "high sulfur content" stating that it is not known what is encompassed by the phrase. New claim 95 does not contain the phrases. However, the applicant submits that the phrases were well defined on page 6, line 21 to page 7, line 4.

In claim 75, 78, 89, and 94 the Examiner objects to the phrase "modified seed storage protein" as indefinite. The phrase has been removed from the claims. Applicants have determined that the phrases "seed storage protein" or "modified seed storage protein" do not properly include many of the plant derived polypeptides

described in the specification. For example hordothionin and chymotrypsin inhibitor are not seed storage proteins.

The Examiner states that "the transformed cell" and "the transformed plant seed" in claims 75 and 76 lack proper antecedent basis. The claims have been rewritten. However, applicant is not aware of any rule or regulation that requires repeating a phrase verbatim. All that is required is that the claim contain no ambiguity, i.e. there is only one transformed cell in the claim.

Claim 79 is objected to as being improperly dependent. The rejection is traversed. It is respectfully submitted that a claim to a transformed seed can reasonably depend from a claim to a method to increase the nutritional value of a cereal plant seed. As noted above claim 75 has been rewritten and recites "A method for increasing the level of lysine or a sulfur-containing amino acid in a cereal plant seed". A dependent claim to the cereal plant seed produced by the method is appropriate.

The Examiner objects to the lack of antecedent basis for "the transformed seed" and "the transformed plant seed" in claims 81, 82, 83, 84, and 85. The claims have been rewritten. However, it is again pointed out that there is no indefiniteness or ambiguity. Proper antecedent basis does not require verbatim repeating of an element.

In claim 89 the Examiner objects to the phrase "elevated level of lysine or methionine" as being indefinite. The claim is rewritten to provide definite ranges.

Claims 75-94 are rejected under 35 U.S.C. 102(e) as being anticipated by Falco et al. (US 5,773,691). The Examiner states that Falco discloses transformed plant seed, including corn, and feed produced from said seed, having enhanced lysine content by introduction of vectors encoding lysine insensitive enzymes or lysine-rich proteins. In particular, Falco teaches said transformed plants wherein the increases in lysine are 10-400%, and Falco teaches use of an endosperm-specific promoter, including the zein promoter, and the use of a gene encoding 2S albumin from Brazil nut. Hence all of the claim limitations have been previously disclosed by Falco.

New claims 95-102 distinguish over Falco et al. by requiring the combination of a cereal plant, expression of a plant derived polypeptide, and an endosperm-preferred promoter. In the Background Falco et al. disclose BNP DNA linked to a phaseolin promoter and expressed in tobacco. Falco et al. further disclose synthetic storage proteins (SSP). These do not appear to be plant derived, Col. 30, line 15-Col. 31, line 62 and Examples 21 and 23. Therefore, Falco et al. do not disclose the specific combination in the present claims, namely a transformed cereal plant and/or a transformed cereal plant seed, a plant derived polypeptide, and an endosperm preferred promoter.

Claims 75-94 are rejected under et U.S.C. 103(a) as being unpatentable over Rao et al. (US 5,885,802) and Rao et al. (US 5,990,389).

As noted by the Examiner the present claims distinguish over Rao et al. by requiring an endosperm-preferred promoter.

The Examiner states that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of Applicant's invention to modify the invention of Rao *et al.* to substitute an endosperm-specific promoter, as admitted by Applicant to have been known in the art, for the constitutive promoter, because the invention was clearly directed to modification of seed tissue, and especially endosperm.

Rao et al. broadly disclose protein engineering. The current claims distinguish over Rao et al. by requiring an endosperm-preferred promoter.

The Examiner further states that "Applicant admits that endosperm-specific promoters, including the zein promoter and the waxy promoter, were well known in the art at the time of Applicant's invention.

Citing references which merely indicate that isolated elements and/or features recited in the claims are known is not a sufficient basis for concluding that the combination of claimed elements would have been obvious. See *Ex parte Hiyamizu* (BPAI 1988) 10 PQ2d 1393.

The Examiner states that the promoters are functional equivalents, and it would have been obvious to substitute one functional equivalent for another.

It is submitted that constitutive expression is not the functional equivalent of endosperm-specific expression. Constitutive expression of a protein can have adverse affects on the growth and development of the plant.

The Examiner notes that Hordothionine is a seed protein and hence expression in the seed (the major portion of which is endosperm) would be expected to be successful.

The Examiner has provided no motivation to express plant-derived proteins in the endosperm of cereal plant seed. The Examiner suggests that it could be done, however, that is not the standard. In the absence of a suggestion or motivation to combine the elements of the claim, a *prima facie* case of obviousness is not shown. As the Federal Circuit stated in the case of *In re O'Farrell:*

> "...what was obvious-to-try" was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention of how to achieve it." 853 F. 2d at 903, 7 USPQ 2d at 1681.

Thus, obvious-to-try is not a proper basis for rejecting the claims under 35 U.S.C. §103 because there is no suggestion or expressed expectation of success in the prior art that would have led one to perform the experimentation in the first place.

Further, there must also be some reasonable expectation of success relating to the prior art that must make any proposed modification or changes in the prior art obvious to do rather than obvious to try.

It is noted that Falco et al. discloses that "No increase in free lysine was observed in seed expressing *Corynebacterium* DHDPS plus *E. coli* from the glutelin 2 promoter with or without AKIII-M4". Falco et al. further indicates that "lysine catabolism is expected to be much greater in the endosperm than the embryo and this probably prevents the accumulation of increased levels of lysine in seeds expressing Corynebacterium DHDPS plus E. coli AKIII-M4 from the glutelin 2 promoter".

Based on the disclosure of Falco et al. one skilled in the art would not have a reasonable expectation of success.

Claims 75-94 are rejected under 35 U.S.C. 103(a) as being unpatentable over Jaynes et al. (US 5,811,654) in view of Applicants Admission. This rejection is repeated for the reasons of record as set forth in the Official action mailed 8/9/00 as applied to claims 57-74, and the Official action mailed 4/21/00 as applied to Claims 36-56. The rejection is repeated below.

The Examiner states that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of Applicant's invention to modify the invention of Jaynes to direct expression of the modified gene to the endosperm by expressing it behind an endosperm-specific promoter as admitted by Applicant to have been well know in the art. It was well known in the art that increased nutritional value of seed (the major portion of which in endosperm tissue) was particularly desirable given the importance of grains as a food source in the impoverished regions of the world. One would have had a reasonable expectation of success in view of the success of Jaynes.

There is no motivation or suggestion to modify the invention of Jaynes et al. to direct expression of plant derived polynucleotide to the endosperm. The fact that endosperm-preferred promoters were known in the art does not arise to the level of a suggestion or motivation to combine them with polynucleotides that increase the level of lysine or a sulfur-containing amino acid. The mere fact that references can be combined does not render the resultant combination obvious unless the prior art also suggest the desirability of the combination. There must be some suggestion of the desirability of the combination. None is found in the reference. See *ACS Hospital Systems, Inc. v. Montefiore Hospital* (221 USPQ 929).

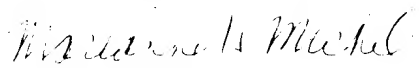
Further both the suggestion to make the claimed composition or device or carry out the claimed process and the reasonable expectation of success must be founded in the prior art, not in applicant's disclosure. See *In re Vaeck* (20 USPQ2d 1438).

Based on the teachings of Falco et al. one skilled in the art would be discouraged from expressing a protein in the endosperm because of lysine catabolism. An invention cannot be obvious when one of the references teaches away from the claimed invention. See *In re Grasseli* 218 USPQ 769.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

In view of the above comments and amendments, reconsideration and allowance of the remaining claims is respectfully requested.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

Claims 75, 80-89, and 94 have been canceled without prejudice or disclaimer.

Claims 76, 77, 78, 79, 90, 91, 92 and 93 have been amended as follows:

76. (Amended) The method of claim 95 ~~75~~ wherein the transformed cereal plant seed is from maize, wheat, rice, or sorghum.

77. (Amended) The method of claim 76 wherein the transformed cereal plant seed is from maize or sorghum.

78. (Amended) The method of claim 95 ~~75~~ wherein the plant derived polynucleotide encodes ~~barley alpha hordothionin or soybean 2S albumin protein or modified proteins of same~~ HT12 or ESA.

79. (Amended) A transformed cereal plant seed produced by the method of claim 95 ~~75~~.

90. (Amended) The expression cassette according to claim 104 ~~89~~ wherein the promoter is a gamma zein promoter or a waxy promoter.

91. (Amended) A vector comprising the expression cassette of claim 104 ~~89~~.

92. (Amended) A cereal plant cell transformed with the vector of claim 91.

93. (Amended) A transformed cereal plant comprising the vector of claim 91.

New claims 95-111 have been added as follows:

95. A method for increasing the level of lysine or a sulfur-containing amino acid in a cereal plant seed, the method comprises transforming a cereal plant cell with an expression cassette and regenerating a transformed cereal plant to produce a transformed cereal plant seed, wherein the expression cassette comprises a seed endosperm-preferred promoter operably linked to a plant derived polynucleotide encoding a polypeptide, and wherein expression of the polypeptide increases the level of lysine or a sulfur-containing amino acid in the transformed cereal plant seed compared to a corresponding non-transformed cereal plant seed.

96. The method of claim 95 wherein the seed endosperm-preferred promoter is heterologous to the plant derived polynucleotide.

97. A transformed cereal plant seed which has been transformed with a plant derived polynucleotide to express a polypeptide in endosperm of the transformed cereal plant seed, wherein the transformed cereal plant seed exhibits an elevated level of lysine or a sulfur-containing amino acid compared to a corresponding non-transformed cereal plant seed.

98. The transformed cereal plant seed of claim 97 wherein the transformed cereal plant seed is from maize, wheat, rice, or sorghum.

99. The transformed cereal plant seed of claim 98 wherein the transformed cereal plant seed is from maize or sorghum.

100. The transformed cereal plant seed according to claim 97 wherein the amount of lysine or sulfur-containing amino acid in the transformed cereal plant seed is increased at least about 10 percent by weight compared to a corresponding non-transformed cereal plant seed.

101. The transformed cereal plant seed according to claim 100 wherein the amount of lysine or sulfur-containing amino acid in the transformed cereal plant seed is increased at least about 15 percent by weight to about 10 times compared to a corresponding non-transformed cereal plant seed.

102. The transformed cereal plant seed according to claim 101 wherein the amount of lysine or sulfur-containing amino acid in the transformed cereal plant seed is increased at least about 20 percent by weight to about 10 times compared to a corresponding non-transformed cereal plant seed.

103. A food or feed product produced from the transformed cereal plant seed of claim 97.

104. An expression cassette comprising a seed endosperm-preferred promoter operably linked to a plant derived polynucleotide encoding a polypeptide having about 7 mole % to about 50 mole % lysine or about 6 mole % to about 40 mole % of a sulfur containing amino acid.

105. The expression cassette of claim 104 wherein the seed endosperm-preferred promoter is heterologous to the plant derived polynucleotide.

106. A seed from a transformed cereal plant which has been transformed with a plant derived polynucleotide to express a polypeptide in the endosperm of the transformed cereal plant seed, wherein the transformed cereal plant seed exhibits an elevated level of lysine or a sulfur-containing amino acid compared to a corresponding non-transformed cereal plant seed.

107. A method for increasing the level of lysine or a sulfur-containing amino acid in a maize seed, the method comprises transforming a maize cell with an expression cassette and regenerating a transformed maize plant to produce a

transformed maize seed, wherein the expression cassette comprises a seed endosperm-preferred promoter operably linked to a plant derived polynucleotide encoding a polypeptide, and wherein expression of the polypeptide increases the level of lysine or a sulfur-containing amino acid in seed of the transformed maize plant compared to seed of a corresponding non-transformed maize plant.

108. The method of claim 107 wherein the seed endosperm-preferred promoter is heterologous to the plant derived polynucleotide.

109. A transformed maize seed, the endosperm of which contains an elevated level of lysine or a sulfur-containing amino acid compared to a corresponding non-transformed maize seed.

110. A transformed maize seed which has been transformed with a plant derived polynucleotide to express a polypeptide in the endosperm of the transformed maize seed, wherein the transformed maize seed exhibits an elevated level of lysine or a sulfur-containing amino acid compared to a corresponding non-transformed maize seed.

111. A food or feed product produced from the transformed cereal plant seed of claim 109.